Association of genetic variants and outcomes in non-ischemic dilated cardiomyopathy

Brief title: Genetics and prognosis in DCM

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Background: The clinical relevance of genetic variants in non-ischemic dilated cardiomyopathy (DCM) is unsettled.

Objectives: This study sought to assess the prognostic impact of disease-causing genetic variants in DCM.

Methods: Baseline and longitudinal clinical data from 1005 genotyped DCM probands were retrospectively collected at 20 centers. 372 (37%) patients had pathogenic/likely pathogenic variants (genotype-positive) and 633 (63%) were genotype-negative. The primary endpoint was a composite of major adverse cardiovascular events (MACE). Secondary endpoints were end-stage heart failure (ESHF), malignant ventricular arrhythmia (MVA) and left ventricular reverse remodeling (LVRR).

Results: After a median follow-up of 4.0 years (IQR 1.7-7.5), the primary endpoint had occurred in 118 patients (31.7%) in the genotype-positive group and in 125 patients (19.8%) in the genotype-negative group (hazard ratio [HR], 1.51; 95% confidence interval [CI], 1.17 to 1.94; P=0.001). ESHF occurred in 60 genotype-positive patients (16.1%) and in 55 genotype-negative patients (8.7%) (HR 1.67; 95%CI, 1.16 to 2.41; P=0.006). MVA occurred in 73 genotype-positive patients (19.6%) and in 77 genotype-negative patients (12.2%) (HR 1.50; 95%CI, 1.09 to 2.07; P=0.013). LVRR occurred in 39.6% in the genotype-positive group and in 46.2% in the genotype-negative group (P=0.047). Among individuals with baseline LVEF ≤35%, genotype-positive patients exhibited more MACE, ESHF and MVA than genotype-negative peers (all P<0.02). LVRR and clinical outcomes varied depending on the underlying affected gene.

Conclusions: In this study, DCM patients with pathogenic/likely pathogenic variants had worse prognosis than genotype-negative individuals. Clinical course differed depending on the underlying affected gene.

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Key Words: Dilated cardiomyopathy, genetics, mutation, prognosis, sudden cardiac death, heart failure, ventricular arrhythmia, left ventricular reverse remodeling.

Condensed abstract

This study sought to assess the prognostic impact of disease-causing genetic variants in DCM. Baseline and longitudinal clinical data from 1005 genotyped DCM probands were retrospectively collected at 20 centers. 372 patients had pathogenic/likely pathogenic variants (genotype-positive) and 633 were genotype-negative. After a median follow-up of 4.0 years, MACE, end-stage heart failure and major ventricular arrhythmias occurred more frequently in genotype-positive than in genotype-negative individuals (all $P<0.01$). Left ventricle reverse remodeling (LVRR) occurred in 39.6% in the genotype-positive group and in 46.2% in the genotype-negative group ($P=0.047$). LVRR and clinical events varied depending on the underlying affected gene.

Condensed abstract word count: 98

Abbreviations:

ARB: Angiotensin Receptor Blockers

ESC: European Society of Cardiology

DCM: Dilated Cardiomyopathy

MACE: Major Adverse Cardiovascular Event

ESHF: End-Stage Heart Failure

MVA Malignant Ventricular Arrhythmia

LVRR: Left Ventricular Reverse Remodeling

LVEF: Left Ventricular Ejection Fraction

Introduction

Non-ischemic dilated cardiomyopathy (DCM) is characterized by left ventricular (LV) enlargement and systolic dysfunction that cannot be attributed to abnormal loading conditions or to coronary artery disease. It has an estimated population prevalence of 1:250 to 1:2500 and is the most frequent cause of heart failure in the young and the leading cause of heart transplantation worldwide. DCM constitutes a common substrate for ventricular arrhythmias and is associated with a higher risk of sudden cardiac death (SCD) (1, 2).

In up to 40-50% of patients, DCM is inherited as a Mendelian trait caused by genetic variants in >40 genes that encode a heterogeneous group of proteins. Such genetic heterogeneity likely contributes to the variable phenotypes and expressivity observed in DCM. Indeed, there is growing evidence that the clinical course depends on the underlying affected gene (2–5).

With the exception of DCM caused by genetic variants in *TTN* and *LMNA* genes (6–9), our understanding of the natural history of genetic DCM is poor. Comprehensive information on the clinical impact of genetic findings in DCM is limited and data from large cohorts are not available (2). Accordingly, the present study sought to assess the clinical impact of genotype findings on prognosis in a large multicenter cohort of patients with non-ischemic DCM.

Methods

Study population

This was a multicenter, retrospective, observational, and longitudinal study of consecutive genetically-evaluated probands with DCM recruited from inherited cardiac diseases and heart failure units at 20 Spanish hospitals between 2015 and 2020.

DCM was defined as left ventricular ejection fraction (LVEF) <50% on echocardiogram at diagnosis in the absence of abnormal loading conditions, coronary artery disease, excessive alcohol consumption or any other identifiable cause (10). Only patients over the age of 15 at the time of diagnosis were included. Participating individuals had been genetically tested using targeted next-generation sequencing (NGS) panels at participating institutions or at an accredited genetics laboratory with no *a priori* selection based on family history of DCM or clinical phenotype. Although the NGS panels could differ in the number of genes, all included >50 genes related to cardiomyopathies.

Most of the centers had inherited cardiac diseases programs and followed the recommendations of the Spanish Society of Cardiology (11). On the first contact, familial data were obtained after a structured interview and a family pedigree was drawn. Clinical screening and cascade genetic screening (if pathogenic or likely pathogenic variant were identified) were offered to relatives.

Although the study primary cohort included only unrelated DCM probands, consecutive relatives with DCM (n=156) who harbored a pathogenic or likely pathogenic genetic variant previously identified by NGS panels of >50 genes in a DCM proband were added to the cohort in order to expand the genotype-positive group and analyze clinical outcomes according to genotype and LVEF.

Demographics, symptoms, 12-lead electrocardiogram, and transthoracic echocardiogram (TTE) data at first and last evaluation at participating centers were extracted from clinical records using uniform methodology. DCM was defined as familial if one or more relatives (in addition to the proband) had DCM during life or at postmortem examination; sporadic case was used indistinctly as non-familial DCM, indicating that there was no family history of DCM and no cases of DCM were detected during familial screening in case it

was performed. A relative was considered as dying of DCM if they experienced a SCD or a heart-failure death with a previous diagnosis of DCM.

The study was approved by Hospital Universitario Puerta de Hierro ethics committee and conformed to the principles of the Helsinki Declaration. The authors from each participating center guarantee the integrity of data.

Variant classification

Variants were classified as pathogenic (P), likely pathogenic (LP), unknown significance (VUS), or likely benign/benign (LB/B) after a systematic review by a cardiologist expert in cardiovascular genetics (JPO) using modified criteria of the American College of Medical Genetics (12), as described in Online Supplementary Methods. A variant was considered disease-causing if it affected a DCM-related gene and was classified P/LP. Patients harboring P/LP variants were considered "Genotype-Positive", and patients harboring VUS/LB/B variants were considered "Genotype-Negative" (Online Figure S1). The variants' frequencies in the general population were extracted from the gnomAD database v2.1.1 (13). We also added the information of more than 5,254 index cases with no evidence of structural cardiac disease (channelopathies and aortic diseases) sequenced by NGS in the Health in Code Molecular Genetics Laboratory (A Coruña, Spain) with a library that included all the genes with genotype-positive variants detected in this study. This cohort was used to obtain an ancestry-specific control set, minimizing the likelihood of incorrectly categorizing variants as disease-causing if they were present in Spanish controls.

Genes were clustered into functional gene groups based on similar common functions, involvement in biological processes, localization to subcellular compartments, and other shared properties based on consolidated scientific evidence from the literature and

available biological databases (14). Because of its specific characteristics of frequency in DCM, *TTN* was considered as a separate group. Functional gene groups included the following: (1) structural cytoskeleton-Z disc; (2) desmosomal; (3) nuclear envelope; (4) motor sarcomeric; (5) *TTN*; and, (6) other genes. Individuals with more than one pathogenic or likely pathogenic variant were excluded from the functional gene group analysis to maintain a conservative approach.

Outcomes

The primary endpoint was a composite of major adverse cardiovascular events (MACE), which included end-stage heart failure (ESHF), major ventricular arrhythmias (MVA), and fatal and non-fatal stroke. Secondary endpoints were ESHF, MVA and left ventricular reverse remodeling (LVRR). ESHF included ventricular assist device implantation for refractory heart failure, heart transplant, and ESHF-related mortality. MVA included SCD, aborted SCD, sustained ventricular tachycardia, and appropriate implantable cardioverter-defibrillator (ICD) interventions. LVRR was defined as either LV normalization (LVEF improvement to ≥50% with a ≥5% LVEF increment on TTE at the last follow-up) or an absolute increase in LVEF by ≥10% on TTE at the last follow-up from initial TTE at baseline, as described (6, 15, 16).

All patients had planned reviews every 6-12 months or more frequently if clinically indicated. The follow-up for each patient was calculated from the date of their first evaluation at a participating center, to the occurrence of a study endpoint, death from another cause, or the date of their most recent evaluation.

Statistical analysis

Continuous variables are expressed as means (±SD) or as medians (with interquartile ranges, IQR), as appropriate. Groups were compared using Student's t-test or the Mann-Whitney test, or analysis of variance or the Kruskal-Wallis test when comparing more than two groups. Non-continuous categorical variables are expressed as counts (percentages) and were compared using the chi-square or Fisher's exact test as appropriate. The cumulative probability of an event on follow-up was estimated using the Kaplan-Meier method, and the log-rank test was used to compare survival between groups. To assess the association of genetic status with the primary and secondary endpoints, a univariate Cox regression model (for MACE, ESHF and MVA) and a logistic regression analysis (for LVRR) were applied. Analyses were conducted using Stata Statistics version 16 (StataCorp LLC, Texas). Two-tailed P-values of 0.05 or less defined statistical significance.

Results

A total of 1,005 probands met inclusion criteria. Genetic testing was positive in 372 patients (37.0%); eight of these index cases (0.8%) harbored two disease-causing variants. A complete list of disease-causing variants can be found in Supplemental Material. A VUS was detected in 244 patients (24.3%), whereas genetic study failed to identify any relevant variant in 389 patients (38.7%) (Online Figure S2).

The presence of a positive genetic test in index cases was higher in individuals with familial DCM (47.3%, 226/478) than in sporadic cases (27.7%, 146/527; P<0.001).

Characteristics of the probands

Characteristics of the patients are presented in Table 1. Male sex prevailed (68.5%); median age at diagnosis was 51 (IQR 42-61) years and most patients were in New York Heart Association class I or II (64.4%) at baseline. Mean baseline LVEF was 32.0±10.5% and 63.3% of patients had an LVEF ≤35%. The prevalence of atrial fibrillation was 11.6%; left bundle branch block was present in 33.0% of patients and a third-degree AV block in 2.4%.

Regarding medical treatment, 83.6% of the patients at baseline were treated with β-blockers and 87.1% with an ACE inhibitor (ACEI) or an angiotensin receptor blocker (ARB). At last follow-up, 93.0% of patients were receiving β-blockers, 66.6% ACEI/ARB, 26.7% sacubitril/valsartan and 67.6% aldosterone-receptor antagonists. In relation to device therapy, 425 patients had an ICD (42.3%) and 167 received cardiac resynchronization therapy (16.6%).

Genotype-positive patients were significantly younger at diagnosis than genotype-negative patients (50 [IQR 39.5-58] vs. 52 [IQR 43-62] years; P<0.001) and were more likely to have a family history of SCD (23.1% vs. 16.1%; P=0.006). Baseline

echocardiographic parameters were similar between groups, with no difference in LVEF or the proportion of patients with LVEF ≤35%.

Among the 363 index cases with one pathogenic or likely pathogenic variant, the most frequently involved genes were *TTN*, identified in 141 individuals (38.7%), followed by *LMNA* (31, 8.5%), *DSP* (31, 8.5%), *BAG3* (24, 6.6%), *FLNC* (21, 5.8%), *RBM20* (20, 5.5%) and *MYH7* (17, 4.7%). The distribution of genes according to the functional gene group in probands with a unique P/LP variant and their clinical characteristics are listed in Online Tables S1 and S4.

Characteristics of patients did not differ between functional gene groups, except for a trend towards a family history of SCD and skeletal myopathy in the nuclear envelope group. Atrial fibrillation, left bundle branch block, and complete AV block were also more prevalent in this group (Online Table S4).

Outcomes in probands

After a median follow-up of 4.04 years (IQR, 1.7-7.5), MACE occurred in 243 patients (24.2%), 115 patients (11.4%) had ESHF events, and 150 patients (14.9%) had MVA. Clinical outcomes are presented in Figure 1 and Table 2. MACE occurred in 118 patients (31.7%) in the genotype-positive group and in 125 patients (19.7%) in the genotype-negative group. The hazard ratio (HR) for MACE was 1.51 (95% confidence interval [CI], 1.17 to 1.94; P=0.001) for genotype-positive patients compared with the genotype-negative patients. ESHF occurred in 60 patients (16.1%) in the genotype-positive group and in 55 patients (8.7%) in the genotype-negative group (HR, 1.67; 95%CI, 1.16 to 2.41; P=0.006). MVA occurred in 73 patients (19.6%) in the genotype-positive group and in 77 patients (12.1%) in the genotype-negative (HR, 1.50; 95%CI, 1.09 to 2.07; P=0.013). LVRR occurred in 422 of the 966 probands (43.7%) with serial echocardiograms suitable

for the analysis [median time between baseline and last TTE of 3.93 (IQR 1.78-7.29) years]. LVRR occurred more frequently in genotype-negative patients than in genotype-positive patients (46.2% vs. 39.6%; P=0.047) (Figure 1D and Online Table S5).

Outcomes differed in genotype-positive patients according to the underlying affected functional gene group (Figure 2 and Online Table S6). The worst cumulative incidence of MACE was observed in the nuclear envelope gene group. Patients from this functional gene group also exhibited higher ESHF and MVA than did patients in the remaining functional groups (P<0.001). The desmosomal and cytoskeleton/Z-disk gene groups exhibited a lower risk of MACE and MVA than the nuclear envelope group but higher than the other functional gene groups.

Differences were also noted in LVRR between the functional gene groups (Figure 2D). The *TTN* group had the highest rate of LVRR (53.2%) and the worst response was observed in the desmosomal genes group (11.1%). LVRR of the nuclear envelope, sarcomeric, and cytoskeleton/Z-disk groups was 25.0%, 28.3%, and 42.5%, respectively. When compared with the genotype-negative group, the LVRR rate was lower in the desmosomal, nuclear envelope, and sarcomeric functional groups (all P<0.001).

Characteristics of relatives with DCM

A total of 156 genotype-positive relatives with DCM were identified during cascade screening. Compared with probands, relatives were diagnosed younger (43.5 [IQR 31-57] vs. 51 [IQR 42-61] years; P<0.001) and exhibited a higher LVEF (40.6±9.7 vs. 32.0±10.5) and lower LVEDD (57.0±6.4 vs. 61.4±8.0) at initial evaluation (Online Table S7). Not unexpectedly, relatives had a higher proportion of asymptomatic patients (65.4% vs. 30.5%) with a lower proportion of patients with a LVEF ≤35% (24.4% vs. 63.3%) (all P<0.001).

Although relatives exhibited a milder DCM phenotype, there were no differences in the composite outcomes of MACE, ESHF, and MVA between index cases and relatives, and LVRR was more frequent in probands than in relatives (43.7% vs 25.0%; P<0.001), supporting also that genotype-positive individuals have worse prognosis than genotype-negative patients as all relatives included in the analysis were genotype-positive versus only 37% of probands.

Clinical outcomes in the overall cohort and classification by functional gene groups after expansion of the genotype-positive DCM group by the incorporation of relatives provided similar findings to the proband cohort, with increased MACE, ESHF, and MVA and reduced LVRR in genotype-positive DCM and varied clinical course depending on the underlying affected gene (Figure 3 and online Figure S4).

Events according to the presence of severe systolic cardiac dysfunction

Outcomes in the whole cohort of patients with LVEF ≤35% and >35% are shown in Figure 4. Genotype-positive patients with baseline LVEF ≤35% had a higher incidence of MACE (HR, 1.62; 95%CI, 1.22 to 2.14; P=0.001), ESHF (HR, 1.68; 95%CI, 1.13 to 2.48; P=0.010) and MVA (HR, 1.58; 95%CI, 1.09 to 2.28; P=0.015) than did genotype-negative patients with LVEF ≤35%. By contrast, outcomes in genotype-positive and genotype-negative patients with LVEF >35% were not statistically different.

Discussion

In this large multicenter study of genotyped patients with DCM, we found that those with pathogenic/likely pathogenic variants had a worse clinical outcome than their genotype-negative peers. We also found that genetic testing identifies patients at higher risk of MVA and ESHF when LVEF is ≤35%, and that clinical course varies depending on the affected gene (central illustration).

This study constitutes the largest cohort of genotyped patients with DCM and clinical outcomes data reported to date and illustrates the clinical utility of genetic testing to identify individuals at higher risk of adverse cardiovascular events, namely ESHF and SCD. Pathogenic or likely pathogenic genetic variants were identified in 37.0% of index patients in the cohort, confirming the significant genetic yield in DCM achieved by NGS. The proportion of probands with familial-DCM and the diagnostic yield of genetic testing observed in our cohort was relatively high, likely reflecting that the majority of the participant centers had specific inherited cardiac disease programs. Nevertheless, the diagnostic yield and distribution of genes was in line with other recent cohorts from inherited cardiac diseases centers, with *TTN* being the most frequently affected gene (17, 18).

Although worse outcomes of genetically-caused DCM had been suggested previously (19), this is the first study demonstrating that carrying a DCM mutation is associated with higher risk for clinical endpoints (both arrhythmic and ESHF) compared with genotype-negative patients.

Our study adds to the available body of data to consider formulating the indications for ICD implantation in patients with non-ischemic DCM. Although current clinical practice guidelines recommend LVEF for risk stratification of SCD and for guiding ICD implantation in DCM, this recommendation is still a matter of debate in non-ischemic

DCM (20, 21). In the DANISH trial, prophylactic ICD implantation in patients with non-ischemic DCM with LVEF ≤35% was not associated with improved survival (22). The results of the DANISH trial and subsequent meta-analyses suggest that using LVEF as the sole prognostic factor for predicting SCD is likely inadequate in DCM, and additional factors are needed to identify individuals who would benefit from ICD implantation (23–25).

Our results indicate that carrying a DCM-causing variant is associated with MVA, particularly in patients with LVEF ≤35%. Accordingly, genetic testing can help guide prophylactic ICD implantation in DCM and an ICD should be offered to genotype-positive DCM patients with LVEF ≤35%.

Interestingly, we found important differences between functional gene clusters in terms of arrhythmic risk, with a markedly higher rate of MVA in the nuclear envelope functional group where genetic variants in *LMNA* predominated. These findings are consistent with previous reports showing higher arrhythmic risk in *LMNA*-related DCM (7, 26). The cytoskeleton/Z-disk functional gene group also showed increased arrhythmic risk in our study. Variants in *FLNC* predominated in this group and *FLNC* mutations have also been associated with increased arrhythmic risk (27). Our data confirm these findings and support the recent recommendations of early ICD implantation in patients affected by certain genotypes (28).

Along the same line, we observed a trend towards a higher risk of MVA in genotype-positive patients with LVEF >35%, suggesting that some patients with specific genotypes could benefit from early ICD implantation, as in the case of *LMNA* mutations. Larger genotype-phenotype correlation studies are, however, needed to test this hypothesis and to identify who might benefit from this genetically tailored approach.

Genotype-positive patients also had worse evolution in terms of heart failure course, with a higher incidence of ESHF during follow-up and a worse response to medical treatment, as assessed by LVRR. Again, progression to ESHF and LVRR was not uniform across functional gene groups, opening the door to the adoption of an individualized prediction approach in DCM based on genetic features. In our study, a lower rate of LVRR was observed in desmosomal, nuclear envelope, and sarcomeric gene groups as compared with the better prognosis in the genotype-negative and the remaining gene groups. Interestingly, we did not find impaired reverse remodeling in patients with cytoskeleton-Z disc variants, which contrasts with the findings of Dal Ferro and colleagues (29). The higher number of patients with cytoskeleton-Z disc variants, and stricter criteria applied to define disease-causing variants in our study, likely explains the difference. Additionally, our study confirms previous reports that suggested a more benign clinical course of DCM caused by *TTN* truncating variants, with a lower incidence of ventricular arrhythmias when compared with other genes such as *LMNA* (16, 30). In our study, the *TTN* group showed the lowest incidence of MACE and MVA, and the highest rate of LVRR.

Overall, our results confirm that non-ischemic DCM exhibits a marked phenotypic heterogeneity matched by its genetic heterogeneity, with an inverse relation across genetic groups between risk of adverse events and response to medical therapy assessed by LVRR. This precision medicine approach is particularly relevant when evaluating patients with non-ischemic DCM and systolic dysfunction for whom decisions about referring for heart transplant evaluation or ICD implantation depend on the probability of achieving LVRR or experiencing MVA.

Thus far, DCM treatment has been dominated by a one-fits-all scheme with no or little therapeutic differences based on underlying etiology and genetic characteristics, which

contrasts with what occurs in other areas of medicine such as oncology and hematology when treating certain malignancies. Although genetic testing in DCM is currently not recommended in guidelines (20, 21), we believe that genetic testing and, consequently, a precision medicine approach, should become the standard of care also in cardiology when more data about the impact of genetic features on prognosis become available, and particularly if ongoing clinical trials with drugs directed specifically to certain genetic DCM subtypes show positive results (ClinicalTrials.gov Identifiers: NCT03439514 and NCT04572893).

Future studies will need to address how to incorporate genetic data as a point-of-care tool for physicians and how to integrate this information with additional parameters such as those obtained from advanced imaging techniques, for example, cardiac magnetic resonance.

Study limitations

Limitations of the study include its observational nature and retrospective design. Main DCM genes were evaluated in all cases, but the genes included in NGS-target panels varied between centers and during time, reflecting the changes in the knowledge of DCM genetics in the last five years. Although this is the largest cohort of genotyped DCM patients with complete clinical outcomes published so far, the limited number of patients belonging to some functional gene groups restricts the power of the conclusions about these groups. Furthermore, participating centers were specialized inherited cardiac diseases and heart failure units and, therefore, findings might not be extrapolated to other settings.

Conclusions

Patients with DCM and with pathogenic and likely pathogenic variants had worse prognosis than genotype-negative patients, and clinical course and left ventricle remodeling varied depending on the underlying affected gene.

Clinical perspectives

Competency in Medical Knowledge 1:

Patients with non-ischemic DCM and with pathogenic and likely pathogenic variants have worse prognosis than genotype-negative peers. Genetic testing should be performed in patients with non-ischemic DCM and emerges as a useful tool for risk stratification. Genetic results can be used to predict clinical course and response to medical therapy.

Competency in Medical Knowledge 2:

Arrhythmic risk is high in genotype-positive DCM patients with LVEF ≤35%, supporting that this subset of patients could benefit from ICD implantation.

Translational Outlook:

Because penetrance of DCM-causing mutations is incomplete, further studies are needed to identify the factors that govern the development of DCM in some affected individuals. Future studies also need to address how to combine genetic data with additional parameters such as environmental factors, genetic background, or cardiac imaging findings.

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Figure Legends

Figure 1. Outcomes in genotype-positive versus genotype-negative DCM index patients (n=1005).

Cumulative incidence for composite MACE (top left), ESHF (top right), and MVA (bottom left) and LVRR rate at last follow-up (bottom right) in genotype-positive versus genotype-negative DCM index patients.

Figure 2. Outcomes according to functional gene group in genotype-positive index patients.

Cumulative incidence for composite MACE (top left), ESHF (top right) and MVA (bottom left), and LVRR rate at last follow-up (bottom right) according to functional gene group in genotype-positive DCM index patients. Patients with multiple P/LP variants excluded. * P<0.001 compared with genotype-negative group.

Figure 3. Outcomes in genotype-positive versus genotype-negative patients in the overall cohort.

Cumulative incidence for composite MACE (top left), ESHF (top right), and MVA (bottom left) and LVRR rate at last follow-up (bottom right) in genotype-positive versus genotype-negative DCM patients from the overall cohort (n=1161).

Figure 4. Outcomes according to genotype and LVEF in the overall cohort.

Cumulative incidence for composite MACE, ESHF, and MVA in genotype-positive versus genotype-negative DCM patients with LVEF ≤35% (left) and LVEF >35% (right) at baseline from the overall cohort (n=1161).

Central Illustration. Clinical outcomes in 1005 non-ischemic DCM patients according to genotype. Clinical outcomes of 1005 genotyped DCM index patients were retrospectively collected at 20 centers. Genotype-positive patients exhibited increased MACE, end-stage heart failure and major ventricular arrhythmias and decreased left ventricle reverse remodeling (LVRR) than genotype-positive peers. Clinical outcomes and LVRR varied depending on the underlying affected gene.

Table 1. Characteristics of the patients according to Genetic Results (n=1005).

	Total (N = 1005)	Genotype Positive (N = 372)	Genotype Negative (N = 633)	P value
Demographics				
Male sex (%)	688 (68.46)	248 (66.67)	440 (69.51)	0.349
Median age at diagnosis (IQR), years	51 (42-61)	50 (39.5-58)	52 (43-62)	<0.001
Median age at initial evaluation (IQR), years	53 (44-62)	52 (42-61)	54 (45-64)	0.006
Median follow-up (IQR), years	4.04 (1.7-7.5)	3.96 (2.0-7.1)	4.15 (1.6-7.7)	0.728
FH of DCM (%)	478 (47.56)	226 (60.75)	252 (39.81)	<0.001
FH of SCD 1st degree relative (%)	123 (12.24)	61 (16.40)	62 (9.79)	0.002
FH of SCD non-1st degree relatives (%)	188 (18.71)	86 (23.12)	102 (16.11)	0.006
FH of skeletal myopathy (%)	24 (2.39)	13 (3.49)	11 (1.74)	0.078
Skeletal myopathy (%)*	35 (3.48)	22 (5.91)	13 (2.05)	0.001
Previous SCD (%)	17 (1.69)	4 (1.08)	13 (2.05)	0.245
NYHA III-IV at 1st evaluation (%)	358 (35.62)	146 (39.25)	212 (33.49)	0.066
NYHA at 1st evaluation (%)				0.223
I	306 (30.45)	113 (30.38)	193 (30.49)	
II	341 (33.93)	113 (30.38)	228 (36.02)	
III	304 (30.25)	124 (33.33)	180 (28.44)	
IV	54 (5.37)	22 (5.91)	32 (5.06)	
Baseline ECG				
Atrial fibrillation (%)	116 (11.58)	42 (11.32)	74 (11.73)	0.846
AV block (3rd degree) (%)	24 (2.39)	12 (3.23)	12 (1.90)	0.182
QRS duration, mm	117.84 ± 29.22	109.22 ± 26.76	122.91 ± 29.44	<0.001
LBBB (%)	331 (33.03)	60 (16.17)	271 (42.95)	<0.001
Abnormal T-wave inversion (%)	364 (36.33)	132 (35.58)	232 (36.77)	0.706
Low QRS voltage limb leads (%)	130 (12.97)	87 (23.45)	43 (6.81)	<0.001
Low QRS voltage precordial leads (%)	45 (4.49)	29 (7.82)	16 (2.54)	<0.001
Baseline echocardiogram				
LVEF, %	31.98 ± 10.46	32.17 ± 10.39	31.87 ± 10.51	0.713
LVEF ≤ 35%, (%)	636 (63.28)	233 (62.63)	403 (63.67)	0.743
LVEDD, mm	61.37 ± 8.04	61.05 ± 7.61	61.56 ± 8.28	0.604
MR moderate/severe (%)	339 (34.98)	132 (36.87)	207 (33.88)	0.346
RVSD (any degree) (%)	208 (23.01)	88 (26.91)	120 (20.80)	0.036
Drug treatment at initial evaluation				
Beta-blockers (%)	829 (83.57)	306 (83.38)	523 (83.68)	0.902
ACEIs/ARBs (%)	864 (87.10)	316 (86.10)	548 (87.68)	0.475
Sacubitril/Valsartan (%)	34 (3.43)	14 (3.81)	20 (3.20)	0.607
ARA (%)	447 (45.06)	185 (50.41)	262 (41.92)	0.009
Treatment at last evaluation				
Beta-blocker (%)	923 (93.04)	341 (92.92)	582 (93.12)	0.903
ACEIs/ARBs (%)	661 (66.63)	237 (64.58)	424 (67.84)	0.293
Sacubitril/Valsartan (%)	265 (26.71)	108 (29.43)	157 (25.12)	0.139
ARA (%)	671 (67.64)	263 (71.66)	408 (65.28)	0.038
ICD (%)	425 (42.29)	189 (50.81)	236 (37.28)	<0.001
CRT (%)	167 (16.62)	43 (11.56)	124 (19.59)	0.001

ACEI denotes angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker; ARA, aldosterone-receptor antagonist; AV, atrioventricular; CRT, cardiac resynchronization therapy; DCM, idiopathic dilated cardiomyopathy; FH, family history; ICD, implantable cardioverter defibrillator; LBBB, left bundle branch block; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection

fraction; MR, mitral regurgitation; NYHA, New York Heart Association; RVSD, right ventricular systolic dysfunction; SCD, sudden cardiac death. * Distribution of genotypes available in Online Table S2.

Table 2. Outcomes and Events according to Genetic Results (n=1005).

Clinical events	Total (N = 1005)	Genotype Positive (N = 372)	Genotype Negative (N = 633)	P value
Atrial fibrillation (%)	287 (28.56)	125 (33.60)	162 (25.59)	0.007
Stroke (%)	30 (2.99)	12 (3.23)	18 (2.84)	0.731
Appropriate ICD therapy (%)	93 (9.25)	42 (11.29)	51 (8.06)	0.088
Aborted SCD (%)	31 (3.08)	13 (3.49)	18 (2.84)	0.564
Heart failure hospitalization (%)	338 (33.63)	140 (37.63)	198 (31.28)	0.040
Heart transplant (%)	87 (8.66)	47 (12.63)	40 (6.32)	0.001
LVAD implantation (%)	19 (1.89)	10 (2.69)	9 (1.42)	0.155
All-cause mortality (%)	68 (6.77)	34 (9.14)	34 (5.37)	0.022
HF-related mortality (%)	32 (3.18)	17 (4.57)	15 (2.37)	0.055
MVA-related mortality (%)	14 (1.39)	9 (2.42)	5 (0.79)	0.033
Composite MACE (%)	243 (24.18)	118 (31.72)	125 (19.75)	<0.001
Composite ESHF (%)	115 (11.44)	60 (16.13)	55 (8.69)	<0.001
Composite MVA (%)	150 (14.93)	73 (19.62)	77 (12.16)	0.001
Left ventricular reverse remodeling (%)	422 (43.69)	145 (39.62)	277 (46.17)	0.047

ESHF denotes end-stage heart failure; HF, heart failure; ICD, implantable cardioverter defibrillator; LVAD, left ventricular assist device; MACE, major adverse cardiovascular event; MVA, malignant ventricular arrhythmia; SCD, sudden cardiac death.